APPLICATION OF ALARM PHEROMONE TO TARGETS BY SOUTHERN YELLOWJACKETS (HYMENOPTERA: VESPIDAE)

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Alarm pheromones have been demonstrated for a number of species of social Vespidae including several hornets and yellowjackets (Vespines) (Landolt et al. 1997). Maschwitz (1964a, b) first demonstrated alarm pheromone responses in the yellowjackets Vespula vulgaris L. and V. germanica (Fab.) in response to crushed wasps and body parts. Pheromone-mediated alarm has since been observed in other vespines: Dolichovespula saxonica (Fab.) (Maschwitz 1984), the southern yellowjacket V. squamosa (Drury) (Landolt & Heath 1987, Landolt et al. 1999), the eastern yellowjacket V. maculifrons (Buysson) (Landolt et al. 1995), Provespa anomala Saussure (Maschwitz & Hanel 1988), and Vespa crabro L. (Veith et al. 1984). 2-Methyl-3-butene-2-ol was identified as a component of the alarm pheromone of V. crabro (Veith et al. 1984), and N-3- methylbutylacetamide was isolated and identified as an alarm pheromone of the southern and eastern yellowjackets (Heath & Landolt 1988, Landolt et al. 1995).

The source of alarm pheromones in social wasps generally is the venom, although the head is implicated as an additional source of alarm pheromone for *V. vulgaris* (Aldiss 1983) and *V. squamosa* (Landolt et al. 1999). Alarm behavior in *V. germanica* and *V. vulgaris* occurred in response to the squashed sting apparatus, sting sac, and solvent extract of the sting sac (Maschwitz 1964b) and in *D. saxonica* as a response to crushed venom glands (Maschwitz 1984). Veith et al. (1984) stimulated alarm in *V. crabro* with squashed venom sacs or venom. Landolt & Heath (1987) isolated an alarm pheromone of *V. squamosa* in solvent extracts of the venom sac and glands. Aldiss (1983) observed alarm in *V. vulgaris* in response to crushed conspecific heads, and Landolt et al. (1999) stimulated alarm and attack in the southern yellowjacket with a solvent extract of conspecific heads. Alarm pheromones known in several species of *Polistes* also originate in the venom (reviewed by Landolt et al. 1997).

Despite repeated demonstrations of alarm responses of social wasps to conspecific body parts and extracts of body parts, the alarm signalling process itself remains unknown. We do not know how wasps release alarm pheromone. It is hypothesized that alarm pheromone in venom is released when wasps spray venom or is deposited when wasps sting (Aldiss 1983, Maschwitz 1964b, Greene et al. 1976). An alarm pheromone originating in the head of workers may be released at the mouthparts and applied or evaporated from the mandibles (Landolt et al. 1999).

We report here experimental evidence that an alarm pheromone is deposited on a substrate or target when attacked by southern yellowjackets. We also demonstrated persistence of that alarm pheromone activity that is uncharacteristic of alarm pheromones generally. Alarm pheromones in social insects typically are quite volatile and short-lasting, an advantage in permitting normal colony activities to resume once a threat has passed (Matthews & Matthews 1978).

Preliminary observations that led to this study indicated possible contamination of protective clothing and equipment following attacks by southern yellowjackets. This included seemingly unprovoked responses by yellowjackets to investigators one or more days following other experiments and a residual odor on material and objects

that had been attacked by workers. We conducted experiments to determine if attacking wasps leave a material that elicits alarm and attack in other workers.

Observations and experiments with southern yellowjackets were made in Alachua County, Florida. All testing was done with vigorous underground colonies. The bioassay for these tests involved a cork $(3.7~{\rm cm}\times3.7~{\rm cm})$ connected to a wooden dowel $(3~{\rm m}\log)$ by $1.2~{\rm cm}$ diam.) with 2 interlocking eye hooks screwed into the dowel and cork. The eye hooks permitted movement of the cork on the dowel that made it easier to detect wasp contact with the target. Three colored push pins (red, blue, and green) were stuck into the bottom of the cork. This target (cork with pins) was waved from side to side about $0.3~{\rm m}$ in front of a colony entrance to induce attack from guard workers present in the nest entrance. During these presentations, workers generally attacked the cork as well as the hooks and push pins. During attacks workers made sting thrusts and also appeared to bite the target, with their mandibles open and contacting the cork, hooks, or pins.

An experiment was conducted to determine if freshly attacked targets elicit alarm, as evidence of the deposition of alarm pheromone by wasps onto targets during earlier attacks. Corks were first presented at nest entrances until hit by wasps, with 6-10 wasps contacting the cork. This treated cork was placed in a glass jar in an ice chest and transported to the laboratory and placed in a freezer. This procedure was repeated to accumulate 5 treated corks. A new dowel and cork was used for each replicate of this procedure. A treated cork was subsequently exposed to ambient field conditions for 3 min and was then presented to a second test colony. The cork was moved slowly to $\frac{1}{3}$ m upwind of the colony where it remained for the assay duration. Alarm behavior and hits to the cork were noted for 2 min, with the use of a tape recorder. As a control, an unexposed cork and dowel were presented in the same manner before each assay of a treated cork. Five treated corks were each tested 4 - 5 h apart over several days. Numbers of hits and landings on the five treated corks ranged from 1 to 76 (mean \pm SE = 33.4 ± 30.4), significantly greater than the no hits or landings that occurred on the five control corks (p = 0.036 by Student's t test).

A second experiment was conducted to determine if the alarm activity of material applied by yellowjackets to the cork remained active after exposure at ambient temperatures. The bioassay protocol was the same as above except that each treated cork was aired in the laboratory for 15 h before field tests. The five treated corks were tested over 4 different days, with 2 tested 2.5 h apart on one day. Again, the five control corks elicited no response, with no evidence of alarm and no contacts of yellowjackets with the corks. The 15 h old corks however, elicited alarm and attack to the corks. Numbers of hits and landings ranged from 4 to 83 per assay (mean \pm SE = 35 \pm 33.9), significantly greater than the response to the control (t = 2.33, p = 0.04).

These two experiments demonstrate that alarmed *V. squamosa* apply chemicals to targets that they attack and that such attacked targets may subsequently remain active in eliciting alarm and attack responses from southern yellowjackets. At this time, the source of the pheromone applied is not known. During stinging attacks on the corks, hooks, and pins, venom could have been applied to those surfaces. Also, wasps attacking the targets were seen to bite on the cork, hooks, and pins, with the possibility that other alarm pheromones from gnathal or cephalic glands which open to the mouthparts (Landolt & Akre 1979) could be applied to mark the targets. The southern yellowjacket is known to possess alarm pheromone both in the venom (Landolt & Heath 1987, Heath & Landolt 1988) and in the head (Landolt et al. 1999).

The possible adaptive significance of such a long lasting alarm signal is apparent when considering the functions of sting autotomy in social insects, the lack of sting autotomy in yellowjackets, and the nature of predators of social wasps and bees. Sting autotomy is the loss of the sting and venom sac after a stinging episode, such as occurs in the honey bee, *Apis mellifera* L. (Free 1987) and in the social wasp *Polybioides raphigastra* (Saussure) (Sledge et al. 1999). In both of these species, sting autotomy probably permits the prolonged release of alarm pheromone from the sting apparatus following stinging, marking the intruder and focusing subsequent attacks (Free 1987, Sledge et al. 1999). A similar strategy has been suggested for species of *Apis* (Pickett et al. 1982, Schmidt et al. 1997), including *A. mellifera and Apis cerana* (Fab.), based on the large quantities of eicosenol in the sting apparatus. It is hypothesized that this compound serves as a carrier to prolong the release of more volatile pheromone compounds and to mark an intruder to focus the defending bees. Although the southern yellowjacket does not exhibit sting autotomy, the deposition of a long lasting alarm pheromone during stinging attacks on vertebrate predators may similarly serve to chemically mark the animal. This would focus attacks on the intruder and also alert the colony if and when this predator approached the nest again.

Several vertebrate predators, such as skunks and raccoons, commonly prey on yellowjacket colonies (Akre & Reed 1984). A possible strategy of a vertebrate predator is exemplified by the honey buzzard, a successful nest predator of European yellowjackets (Cobb 1979, cited in Akre & Reed 1984). This bird was persistent in its attacks on excavated subterranean *Vespula* nests over a period of days. The buzzard was driven away from nests repeatedly by wasps, but later reapproached the nest to continue the attack. Such an "attack, retreat, reattack" scenario is a likely strategy for other vertebrate nest predators. Thus, a long lasting alarm pheromone that marks a predator may be highly advantageous to yellowjackets. However, caution must be exercised in interpreting how these results of alarm pheromone persistence from a wooden cork may relate to alarm pheromone on vertebrate skin, fur, or feathers. Additional experimentation with leather or feather targets could help address this question, as would chemical analysis of odors emitted by attacked objects.

SUMMARY

Alarmed southern yellowjacket workers attacking corks placed near colony entrances applied an alarm pheromone that stimulated alarm and attack behavior in another colony 3 min or 15 h after pheromone deposition. Observations of wasps attacking corks indicated deposition or application of alarm pheromone could be made both from the sting and from the mandibles. This long lasting material may serve to mark an attacking vertebrate predator so that it is quickly detected and attacked again upon its return to a wasp colony.

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